Application No. 9/721,507 Filed November 22, 2000 Page 2

In the Specification:

In order to fully comply with 37 C.F.R. 1.821-1.825, please amend the specification as follows.

A. Please delete the paragraph located on page 14, line 26, and substitute therefor the following paragraph:

Figure 6 shows the nucleic acid sequence corresponding to subtilisin E (SEQ ID NO: 4).

B. Please delete the paragraph located on page 35, lines 8-27, and substitute therefor the following paragraph:

In an alternative embodiment of the methods of assembling synthetic and mutagenized gene libraries that are mediated by single-stranded templates, described above, oligonucleotides are synthesized in such a way as to end in a single redundant codon. For example, this is accomplished by first preparing two batches of resin containing either *N-N-G-resin or *N-N-C-resin (where * indicates the attachment end at which new bases are added during synthesis). This can be accomplished using an automated DNA synthesizer according to methods known in the art. For example, a fixed mass (e.g., 10 mg) of *N-N-C is added to the reaction vessel following each trinucleotide coupling set. All subsequent reaction steps are then shared by the progressively accumulated resin. Fresh resin is added after each trinucleotide synthesis step to allow generation of an oligo with a redundancy at each position. As shown in Figure 7A, invariant recombination and digestion sites are optionally incorporated within the backbone structure derived from the oligonucleotide sequences (identified herein as SEQ ID NOs: 5-16). As an alternative to the single base coupling cycle described above, vials containing preformed trinucleotides encoding the amino acid or set of amino acids desired at a given position are optionally included. As shown in Figure 7A, the transfer # indicates the trinucleotide synthesis step at which the progenitor resin is added in order to give the listed sequence. For example, each transfer is optionally transferred to a single synthesis vessel in which the same base is added to each oligonucleotide at each reaction cycle after the redundant codon is incorporated.

C. Please delete the paragraph located on page 144, lines 5-12, and substitute therefor the following paragraph:

Transfer of the library to the pBE shuttle vector, followed by transformation into B. subtilis and selection of antibiotic resistant transformants by growth on nutrient-antibiotic plates allows for

secretory expression and immediate and direct, on-plate measurement of activity and thermostability screening as reported by Zhou et al. (1998), *supra*, using the succinyl-ala-ala-pro-phe-p-nitroanilide (SEQ ID NO: 1) (s-AAPF-pNa) method of Zhou and Arnold (1997), *supra*. This assay allows for rapid assessment of the thermostability of the clones derived from the template-based recombination process.

- **D**. Please delete the paragraph located on page 146, lines 24-30, and substitute therefor the following paragraph:
- a. Oligonucleotide primers PBADGFP3 (P-ATAAGATTAGCGGATCCTAC) (SEQ ID NO: 2) and PBADGFP4 (P-TCGGGCATGGCACTCTTGAA) (SEQ ID NO: 3) which flank the random stop sites in pBAD(Cm)GFP(c3)STOP1 (e.g., 'STOP1 phagemid') were phosphorylated and used to prime amplification of corresponding 500 base pair fragments from the STOP1 and STOP2 phagemids using the TthXL thermostable polymerase mix according to manufacturer's protocol.
- E. Please enter the enclosed paper copy of the Sequence Listing at the end of the application.

In accordance with 37 CFR §1.121 a marked up version of the above-amended paragraphs illustrating the changes introduced by the forgoing amendments are provided in Appendix A.

REMARKS

The above amendments provide identifiers for the sequences contained in the application thus bringing the application into compliance with the sequence listing requirements of 37 C.F.R. 1.821- 1.825. These amendments introduce no new matter. Applicants respectfully request that these amendments be entered. Applicants further submit a complete sequence listing containing sixteen sequences in both paper and Computer readable (electronic) formats.